

<p align="center"><b>22 METHYLPHENIDATE AND RITALINIC ACID QUANTITATION AND CONFIRMATION BY LCMS</b></p>	<p align="center">Page 1 of 4</p>
<p align="center"><b>Division of Forensic Science TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b></p>	<p>Amendment Designator:</p>
	<p>Effective Date: 31-March-2004</p>
<p align="center"><b>22 METHYLPHENIDATE AND RITALINIC ACID QUANTITATION AND CONFIRMATION BY LCMS</b></p> <p><b>22.1 Summary</b></p> <p>22.1.1 Methylphenidate and its metabolite, ritalinic acid, are extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).</p> <p><b>22.2 Specimen Requirements</b></p> <p>22.2.1 One mL blood, urine, gastric or tissue homogenate.</p> <p><b>22.3 Reagents and Standards</b></p> <p>22.3.1 Ammonium acetate</p> <p>22.3.2 Methanol</p> <p>22.3.3 Acetonitrile</p> <p>22.3.4 Methylphenidate, 1 mg/mL</p> <p>22.3.5 Ritalinic acid (<math>\alpha</math>-phenyl-2-piperidineacetic acid), 1 mg/mL</p> <p>22.3.6 Phenacetin, 1 mg/mL</p> <p><b>22.4 Solutions, Internal Standard, Calibrators and Controls</b></p> <p>22.4.1 10 mM Ammonium Acetate: Weight 0.38 g ammonium acetate. Transfer to 500 mL volumetric flask and QS to volume with dH<sub>2</sub>O</p> <p>22.4.2 Working standard solution for methylphenidate and ritalinic acid (0.01 mg/mL)</p> <p>22.4.2.1 Pipet 100 <math>\mu</math>l each of 1 mg/mL stock solutions of methylphenidate and ritalinic acid into a 10 mL volumetric flask and QS to volume with dH<sub>2</sub>O</p> <p>22.4.3 Quality Control (QC) standard solution of methylphenidate and ritalinic acid (0.01 mg/mL)</p> <p>22.4.3.1 Pipet 100 <math>\mu</math>l each of separate 1 mg/mL stock solutions of methylphenidate and ritalinic acid (different manufacturer, lot number or preparation than calibrators) into a 10 mL volumetric flask and QS to volume with dH<sub>2</sub>O</p> <p>22.4.4 Internal standard working solution</p> <p>22.4.4.1 0.1 mg/mL phenacetin: Pipet 1 mL of 1 mg/mL phenacetin stock solution into 10 mL volumetric flask and QS to volume with dH<sub>2</sub>O</p> <p>22.4.5 To prepare the calibration curve, pipet the following volumes of the 1 mg/mL and 0.01 mg/mL methylphenidate and ritalinic acid working standards into appropriately labeled 16 x 125 mm screw cap test tubes. Add 1 mL blank blood to obtain the final concentrations listed below.</p>	

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Concentration of standard (mg/mL)	Amount of Standard (μL)	Final concentration of methylphenidate and ritalinic acid (mg/L)
1 mg/mL	10	10
1 mg/mL	5	5
0.01 mg/mL	200	2
0.01 mg/mL	100	1
0.01 mg/mL	50	0.5
0.01 mg/mL	10	0.1

#### 22.4.6 Controls

##### 22.4.6.1 Methylphenidate and Ritalinic Acid Control

22.4.6.1.1 Pipet 100 μL of the 0.01 mg/mL methylphenidate/ritalinic acid QC solution into an appropriately labeled tube. Add 1 mL blank blood to achieve final concentration of 1 mg/L.

22.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain methylphenidate or ritalinic acid.

#### 22.5 Apparatus

22.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

22.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

22.5.3 Centrifuge capable of 2000-3000 rpm

22.5.4 Nitrogen evaporator with heating block

22.5.5 Vortex mixer

22.5.6 GC autosampler vials with inserts

22.5.7 LC/MS: Agilent Model 1100 LC-MSD

22.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

22.5.7.1.1 Elution conditions:

- 22.5.7.1.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 μM particle size
- 22.5.7.1.1.2 Column thermostat: 30° C
- 22.5.7.1.1.3 Solvent A: 10 mM ammonium acetate in dH<sub>2</sub>O
- 22.5.7.1.1.4 Solvent B: methanol
- 22.5.7.1.1.5 Isocratic elution, stop time: 6.00 min

Time	Solv. B	Flow
0.00	48	0.5

22.5.7.1.2 Spray Chamber

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22.5.7.1.2.1 Ionization Mode: Electrospray  
22.5.7.1.2.2 Gas Temperature: 350° C  
22.5.7.1.2.3 Drying Gas (N<sub>2</sub>): 12.0 L/min  
22.5.7.1.2.4 Nebulizer pressure: 35 psig  
22.5.7.1.2.5 Vcap (Positive): 3500 V

22.5.7.1.3 Selected Ion Monitoring (quantitation ions)

22.5.7.1.3.1 Polarity: Positive  
22.5.7.1.3.2 Injection volume: 1 µL

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0	Ritalinic acid	84	170	1.0	Low	352
		174	170		352	
		<u>220</u>	170		352	
2.3	Phenacetin	110	160	1.0	Low	529
		<u>180</u>	160		529	
3.5	Methylphenidate	84	170	1.0	Low	352
		174	170		352	
		<u>234</u>	170		352	

**22.6 Procedure**

- 22.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.
- 22.6.2 Prepare calibrators and controls.
- 22.6.3 Add 1 mL case specimens to the appropriately labeled tubes.
- 22.6.4 Add 50 µL 0.1 mg/mL phenacetin internal standard working solution to each tube.
- 22.6.5 Slowly, add dropwise 2 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential.
- 22.6.6 Vortex an additional 30 seconds.
- 22.6.7 Place tubes in freezer for at least 30 minutes to facilitate separation.
- 22.6.8 Centrifuge at approximately 2500 rpm for 15 minutes.
- 22.6.9 Transfer top (acetonitrile) layer to clean conical bottom tubes taking care not to transfer any lower layers.
- 22.6.10 Evaporate to dryness at approximately 50° C under nitrogen.
- 22.6.11 Reconstitute samples in 100 µL methanol. Vortex briefly. Transfer to GC microvials and inject on LCMS.

**22.7 Calculation**

- 22.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

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<p><b>22.8 Quality Control and Reporting</b></p> <p>22.8.1 See Toxicology Quality Guidelines</p> <p><b>22.9 References</b></p> <p>22.9.1 J Pearson and R Steiner, in-house development.</p>	